

10/501223

DT04 Rec'd PCT/PTO 12 JUL 2004

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (original) Detoxified and immunogenic mutant of the Tat protein of the HIV-1 virus, characterized in that it comprises at least two mutations in regions 4 and/or 5 of the wild-type Tat protein, and in that, when the mutation is in the region of domain 4, it is in the part delimited by the amino acid in position 49 to the amino acid in position 57, and in that, when the mutation is in domain 5, it is either in the RGD motif, or in the region 88-92, preferably in positions 89 and/or 92, the mutations being mutations by substitution of one amino acid by another.

2. (original) Mutant according to claim 1, characterized in that it comprises at least one mutation in region 4.

3. (currently amended) Mutant according to claim 1 ~~one of claims 1 or 2~~, characterized in that the mutations in domains 4 and/or 5 are capable of conferring at least one of the following properties:

- the cancellation of the transcellular effect of the wild-type Tat protein,
- the alteration of the nuclear localization of the wild-type Tat protein.

4. (currently amended) Mutant according to claim 1 ~~one of claims 1 to 3~~, characterized in that it comprises an additional mutation capable of conferring a loss of the transactivating activity of the wild-type Tat protein.

5. Mutant according to claim 1 ~~[[claims 1 to 4]]~~, characterized in that it comprises a mutation in the N-terminal region of domain 4 of the wild-type Tat protein in the part delimited by the amino acid in position 49 to the amino acid in position 55.

6. (currently amended) Mutant according to claim 1 ~~one of claims 1 to 5~~, characterized in that it comprises a mutation in domain 2 of the wild-type Tat protein, in particular the replacement of any one of the cysteines, advantageously by a serine.

7. (currently amended) Mutant according to claim 1 ~~one of claims 1 to 6~~, characterized in that it comprises at least one of the following mutations:

- replacement in position 27 of a cysteine by a serine,
- replacement in position 51 of a lysine by a threonine,
- replacement in position 52 of an arginine by a leucine,
- replacement in position 55 of an arginine by a leucine,
- replacement in position 57 of an arginine by a leucine,
- replacement in position 79 of a glycine by an alanine,
- replacement in position 89 of a lysine by a leucine,
- replacement in position 92 of a glutamic acid by a glutamine.

8. (currently amended) Mutant according to claim 1 ~~one of claims 1 to 7~~, characterized in that it is chosen from the mutants having two mutations as indicated hereafter, each of the mutations being represented by a triplet: letter-figure-letter, the figure of which indicates the position of the mutated amino acid, the letter preceding the figure corresponds to the amino acid to which the mutation relates and the letter following the

figure corresponds to the amino acid replacing the amino acid preceding the figure:

K51T-R52L (SEQ ID NO: 2)
 K51T-R55L (SEQ ID NO: 3)
 K51T-R57L (SEQ ID NO: 4)
 K51T-G79A (SEQ ID NO: 5)
 K51T-K89L (SEQ ID NO: 6)
 K51T-E92Q (SEQ ID NO: 7)
 R52L-R55L (SEQ ID NO: 8)
 (R52L-R57L (SEQ ID NO: 9)
 R52L-G79A (SEQ ID NO: 10)
 R52L-K89L (SEQ ID NO: 11)
 R52L-E92Q (SEQ ID NO: 12)
 R55L-R57L (SEQ ID NO: 13)
 R55L-G79A (SEQ ID NO: 14)
 R55L-K89L (SEQ ID NO: 15)
 R55L-E92Q (SEQ ID NO: 16)
 R57L-G79A (SEQ ID NO: 17)
 R57L-K89L (SEQ ID NO: 18)
 R57L-E92Q (SEQ ID NO: 19)
 G79A-K89L (SEQ ID NO: 20)
 G79A-E92Q (SEQ ID NO: 21)
 K89L-E92Q (SEQ ID NO: 22).

9. (currently amended) Mutant according to claim 8, characterized in that it is chosen from the following mutants:

K51T-R55L (SEQ ID NO: 3)
 R52L-R55L (SEQ ID NO: 8)
 R52L-G79A (SEQ ID NO: 10)
 R55L-R57L (SEQ ID NO: 13)
 G79A-K89L (SEQ ID NO: 20).

10. (currently amended) Mutant according to claim 1 ~~one of~~
~~claims 1 to 7~~, characterized in that it is chosen from the

mutants having three mutations as indicated hereafter, each of the mutations being represented by a triplet: letter-figure-letter, the figure of which indicates the position of the mutated amino acid, the letter preceding the figure corresponds to the amino acid to which the mutation relates and the letter following the figure corresponds to the amino acid replacing the amino acid preceding the figure:

C27S-K51T-R52L (SEQ ID NO: 23)
 C27S-K51T-R55L (SEQ ID NO: 24)
 C27S-K51T-R57L (SEQ ID NO: 25)
 C27S-K51T-G79A (SEQ ID NO: 26)
 C27S-K51T-K89L (SEQ ID NO: 27)
 C27S-K51T-E92Q (SEQ ID NO: 28)
 C27S-R52L-R55L (SEQ ID NO: 29)
 C27S-R52L-R57L (SEQ ID NO: 30)
 C27S-R52L-G79A (SEQ ID NO: 31)
 C27S-R52L-K89L (SEQ ID NO: 32)
 C27S-R52L-E92Q (SEQ ID NO: 33)
 C27S-R55L-R57L (SEQ ID NO: 34)
 C27S-R55L-G79A (SEQ ID NO: 35)
 C27S-R55L-K89L (SEQ ID NO: 36)
 C27S-R55L-E92Q (SEQ ID NO: 37)
 C27S-R57L-G79A (SEQ ID NO: 38)
 C27S-R57L-K89L (SEQ ID NO: 39)
 C27S-R57L-E92Q (SEQ ID NO: 40)
 C27S-G79A-K89L (SEQ ID NO: 41)
 C27S-G79A-E92Q (SEQ ID NO: 42)
 C27S-K89L-E92Q (SEQ ID NO: 43).

11. (currently amended) Mutant according to claim 10, characterized in that it is chosen from the following mutants:

C27S-K51T-R55L (SEQ ID NO: 24)
 C27S-R52L-R55L (SEQ ID NO: 29)
 C27S-R52L-G79A (SEQ ID NO: 31).

12. (currently amended) Mutant according to claim 1 ~~one of claims 1 to 7~~, characterized in that it is chosen from the mutants having four mutations as indicated hereafter, each of the mutations being represented by a triplet: letter-figure-letter, the figure of which indicates the position of the mutated amino acid, the letter preceding the figure corresponds to the amino acid to which the mutation relates and the letter following the figure corresponds to the amino acid replacing the amino acid preceding the figure:

C27S-K51T-R52L-G79A (SEQ ID NO: 44)
C27S-K51T-R52L-K89L (SEQ ID NO: 45)
C27S-K51T-R52L-E92Q (SEQ ID NO: 46)
C27S-K51T-R55L-G79A (SEQ ID NO: 47)
C27S-K51T-R55L-K89L (SEQ ID NO: 48)
C27S-K51T-R55L-E92Q (SEQ ID NO: 49)
C27S-K51T-R57L-G79A (SEQ ID NO: 50)
C27S-K51T-R57L-K89L (SEQ ID NO: 51)
C27S-K51T-R57L-E92Q (SEQ ID NO: 52)
C27S-K51T-G79A-K89L (SEQ ID NO: 53)
C27S-K51T-G79A-E92Q (SEQ ID NO: 54)
C27S-K51T-K89L-E92Q (SEQ ID NO: 55)
C27S-R52L-G79A-K89L (SEQ ID NO: 56)
C27S-R52L-G79A-E92Q (SEQ ID NO: 57)
C27S-R52L-K89L-E92Q (SEQ ID NO: 58)
C27S-R52L-R55L-G79A (SEQ ID NO: 59)
C27S-R52L-R55L-K89L (SEQ ID NO: 60)
C27S-R52L-R55L-E92Q (SEQ ID NO: 61)
C27S-R52L-R57L-G79A (SEQ ID NO: 62)
C27S-R52L-R57L-K89L (SEQ ID NO: 63)
C27S-R52L-R57L-E92Q (SEQ ID NO: 64)
C27S-R55L-G79A-K89L (SEQ ID NO: 65)
C27S-R55L-G79A-E92Q (SEQ ID NO: 66)
C27S-R55L-K89L-E92Q (SEQ ID NO: 67)

C27S-R55L-R57L-G79A (SEQ ID NO: 68)
 C27S-R55L-R57L-K89L (SEQ ID NO: 69)
 C27S-R55L-R57L-E92Q (SEQ ID NO: 70)
 C27S-R57L-G79A-K89L (SEQ ID NO: 71)
 C27S-R57L-G79A-E92Q (SEQ ID NO: 72)
 C27S-R57L-K89L-E92Q (SEQ ID NO: 73)
 C27S-G79A-K89L-E92Q (SEQ ID NO: 74).

13. (currently amended) Mutant according to claim 12, characterized in that it is chosen from the following mutants:

C27S-K51T-R55L-G79A (SEQ ID NO: 47)
 C27S-K51T-R55L-K89L (SEQ ID NO: 48)
 C27S-K51T-R55L-E92Q (SEQ ID NO: 49)
 C27S-R52L-R55L-G79A (SEQ ID NO: 59).

14. (currently amended) Mutant according to claim 1 ~~one of claims 1 to 7~~, characterized in that it is chosen from the mutants having five mutations as indicated hereafter, each of the mutations being represented by a triplet: letter-figure-letter, the figure of which indicates the position of the mutated amino acid, the letter preceding the figure corresponds to the amino acid to which the mutation relates and the letter following the figure corresponds to the amino acid replacing the amino acid preceding the figure:

C27S-K51T-G79A-K89L-E92Q (SEQ ID NO: 75)
 C27S-K51T-R52L-R55L-G79A (SEQ ID NO: 76)
 C27S-K51T-R52L-R55L-K89L (SEQ ID NO: 77)
 C27S-K51T-R52L-R55L-E92Q (SEQ ID NO: 78)
 C27S-K51T-R52L-R57L-G79A (SEQ ID NO: 79)
 C27S-K51T-R52L-R57L-K89L (SEQ ID NO: 80)
 C27S-K51T-R52L-R57L-E92Q (SEQ ID NO: 81)
 C27S-K51T-R52L-G79A-K89L (SEQ ID NO: 82)
 C27S-K51T-R52L-G79A-E92Q (SEQ ID NO: 83)
 C27S-K51T-R52L-K89L-E92Q (SEQ ID NO: 84)

C27S-K51T-R55L-R57L-G79A (SEQ ID NO: 85)
 C27S-K51T-R55L-R57L-K89L (SEQ ID NO: 86)
 C27S-K51T-R55L-R57L-E92Q (SEQ ID NO: 87)
 C27S-K51T-R55L-G79A-K89L (SEQ ID NO: 88)
 C27S-K51T-R55L-G79A-E92Q (SEQ ID NO: 89)
 C27S-K51T-R55L-K89L-E92Q (SEQ ID NO: 90)
 C27S-K51T-R57L-G79A-K89L (SEQ ID NO: 91)
 C27S-K51T-R57L-G79A-E92Q (SEQ ID NO: 92)
 C27S-K51T-R57L-K89L-E92Q (SEQ ID NO: 93)
 C27S-R52L-R55L-R57L-G79A (SEQ ID NO: 94)
 C27S-R52L-R55L-R57L-K89L (SEQ ID NO: 95)
 C27S-R52L-R55L-R57L-E92Q (SEQ ID NO: 96)
 C27S-R52L-R55L-G79A-K89L (SEQ ID NO: 97)
 C27S-R52L-R55L-G79A-E92Q (SEQ ID NO: 98)
 C27S-R52L-R55L-K89L-E92Q (SEQ ID NO: 99)
 C27S-R52L-R57L-G79A-K89L (SEQ ID NO: 100)
 C27S-R52L-R57L-G79A-E92Q (SEQ ID NO: 101)
 C27S-R52L-R57L-K89L-E92Q (SEQ ID NO: 102)
 C27S-R52L-G79A-K89L-E92Q (SEQ ID NO: 103)
 C27S-R55L-R57L-G79A-K89L (SEQ ID NO: 104)
 C27S-R55L-R57L-G79A-E92Q (SEQ ID NO: 105)
 C27S-R55L-R57L-K89L-E92Q (SEQ ID NO: 106)
 C27S-R55L-G79A-K89L-E92Q (SEQ ID NO: 107)
 C27S-R57L-G79A-K89L-E92Q (SEQ ID NO: 108).

15. (currently amended) Mutant according to claim 14, characterized in that it is chosen from the following mutants:

C27S-K51T-R55L-G79A-K89L (SEQ ID NO: 88)
 C27S-K51T-R55L-G79A-E92Q (SEQ ID NO: 89).

16. (currently amended) Nucleotide sequences coding for one of the mutants according to claim 1 ~~one of claims 1 to 15~~.

17. (original) Cell line transfected with a nucleotide sequence according to claim 16.

18. (currently amended) Antibodies directed against one of the mutants according to claim 1 ~~one of claims 1 to 15~~, not recognizing domain D1 of the wild-type protein.

19. (original) Antibodies according to claim 18, recognizing the wild-type protein.

20. (original) Antibodies according to claim 18, not recognizing the wild-type protein.

21. (currently amended) Pharmaceutical composition, in particular a vaccine, containing as active ingredient at least one of the mutants according to claim 1 ~~one of claims 1 to 15~~, or ~~at least one of the nucleotide sequences according to claim 16~~, placed under the control of elements necessary to a constitutive expression of one of the mutants ~~according to one of claims 1 to 15~~, or ~~at least one of the antibodies according to one of claims 18 to 20~~, in combination with a pharmaceutically suitable vehicle.

22. (currently amended) Diagnostic composition for the detection and/or quantification of HIV-1 virus comprising at least one mutant as defined in claim 1 ~~one of claims 1 to 15~~ or ~~at least one antibody according to one of claims 18 to 20~~.

23. (currently amended) Process for the detection and/or quantification of the HIV-1 virus in a biological sample taken from an individual capable of being infected with HIV-1, such as plasma, serum or tissue, characterized in that it comprises stages consisting of:

- bringing said biological sample into contact with a diagnostic composition comprising a mutant as defined in claim 1 ~~one of claims 1 to 15 or an antibody as defined in one of claims 18 to 20~~, under predetermined conditions which allow, if necessary, the formation of antibody/antigen complexes between the mutant defined above and antibodies directed against the wild-type Tat protein or between the antibodies defined above and the wild-type Tat protein, and

- detecting and/or quantifying the formation of said complexes by any appropriate means.

24. (currently amended) Use of Method for the *in vitro* diagnosis of the HIV-1 virus in a biological specimen or sample, which comprises using at least one mutant as defined in claim 1 ~~one of claims 1 to 15 or at least one antibody according to one of claims 18 to 20, for the *in vitro* diagnosis of the HIV-1 virus in a biological specimen or sample.~~

25. (currently amended) Use of Method for the preparation of a vaccine composition, which comprises using at least one mutant as defined in claim 1 ~~one of claims 1 to 15 or at least one antibody according to one of claims 18 to 20, for the preparation of a vaccine composition.~~